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DNA TOPOISOMERASE II INHIBITION BY SUBSTITUTED 1,2,3,4-TETRAHYDRO-β-CARBOLINE DERIVATIVES

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ABSTRACT: A series of 1-aryl-1,2,3,4-tetrahydro-β-carbolines were synthesized, as "truncated" analogs of the novel agent, azatoxin, and assayed *in vitro* for their ability to induce protein-associated DNA strand breaks in the presence of DNA and human DNA topoisomerase II.

DNA topoisomerase II (TPII) is the target for a wide range of cancer chemotherapeutic agents.[1-4] These agents, which include the anthracyclines, amsacrines, ellipticines and the epipodophyllotoxins, possess a considerable diversity in structure, but universally act by forming a ternary complex between DNA, TPII and the drug. This complex, often termed the "cleavable complex", has an enzyme-bound single- or double-stranded "nick" in the DNA and appears to be closely related in structure to an intermediate along the normal catalytic pathway, which the drug has "trapped" and stabilized. We have previously proposed a model for the composite pharmacophore of all TPII-directed agents.[5,6] This model postulates three *sub*domains: 1. an aromatic domain proposed to be involved in DNA intercalation or "intercalation-like" DNA association; 2. a substituent appended to the planar domain, which has a hydrogen bonding functionality ≈5.5 Å *below* the plane of the intercalation domain and proposed to interact with the DNA minor groove; and 3. a domain of considerable structural diversity, which extends *above* the intercalation region and is also postulated to lie in the DNA minor groove.

Although examination of the agents encompassed by this pharmacophore model suggests

that not all *sub*domains are required for activity, it is mechanistically implicit in the model that upon DNA association proximate to an enzyme cleavage site, the drug induces and/or stabilizes a DNA conformation resembling the endogenous enzyme-DNA "cleavable complex" structure. In efforts to further refine our pharmacophore model, we have been engaged in the synthesis of hybrid molecules composed of subunits of existing DNA-TPII active agents. These studies recently produced azatoxin **1**,[7,8] a unique TPII-directed agent that represents a hybrid of the ellipticine and podophyllotoxin nuclei. We report here the structure-activity relationships for a series of "truncated" or *seco*-azatoxin analogs encompassed in the 1-aryl-1,2,3,4-tetrahydro-β-carboline framework **2**.

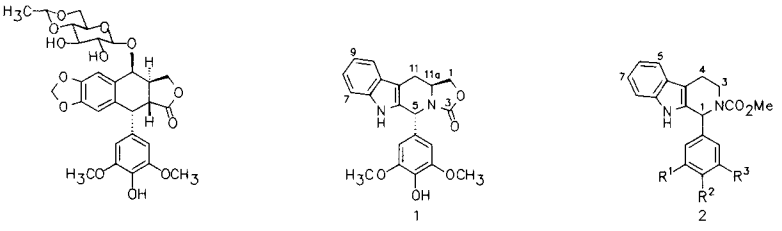


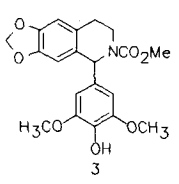
Figure 1. The structures of etoposide, azatoxin **1** and the 1-aryl-tetrahydro-β-carboline derivatives **2**.

The aryl *tetra*hydro-β-carboline derivatives **2** (Figure 1) were designed as conformationally mobile analogs of azatoxin **1**. These compounds were considered to be able to address several important features of the pharmacophore model including: a comparison of aromatic functionality in the DNA association domain, i. e. indole (as in azatoxin **1**) versus methylenedioxyphenyl (as in the epipodophyllotoxins); an assessment of the role of the dihedral angle between the two aromatic regions in TPII-mediated activity; and the facile determination of the SAR for a series of pendant groups.

Compounds **3-9** (Figure 2) were synthesized from the 2-(aryl)ethyl amine *via* a Pictet-Spengler cyclization, according to the method of Bailey[9], followed by carbamoylation with methyl chloroformate in pyridine. The compounds were assayed for their ability to induce cleavable complex formation in the presence of pUC18 plasmid DNA and human placental DNA topoisomerase II[10] using the procedure described by Liu *et al.*[11] (Table). Considerable care must be exercised in the assay of these β-carboline derivatives to ensure the solubility of the agent during the course of the investigation. Figure 3 presents the sequence selectivity data for a selected subset of the most active compounds in this series.

The most active compounds were found to be 1-indolyl-β-carbolines derivative **4** and **5** and the 1-methylenedioxyphenyl-β-carboline **3** (Table). These compounds were found to exhibit stimulation of cleavage at concentrations as low as 25 μ**M** (Figure 3). From our prior study of azatoxin **1** SAR, it is known that TPII-mediated cleavage activity resides in a single enantiomer of

1 (as depicted in Figure 1)[7]. Since the β-carboline derivatives were synthesized as racemates, it would be anticipated that a single enantiomer (*1R*) would exhibit most (or all) TPII activity. By analogy with the (*5S, 11aR*) enantiomer of azatoxin **1**, which shows *no activity* in TPII assays (as contrasted with the potential for *weak activity* or *antagonism* toward the activity of (*5R, 11aS*)-**1**), the (*1S*) enantiomers of β-carbolines **3**, **4**, and **5** would be expected to be *inactive*.



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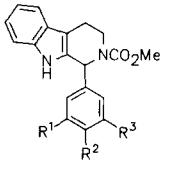

4: $R^1=R^3=OCH_3; R^2=OH$
5: $R^1=OCH_3; R^2=OH; R^3=OCH_3$
6: $R^1=R^3=H; R^2=OH$
7: $R^1=R^2=R^3=OCH_3$
8: $R^1=H; R^2=NHSO_2CH_3; R^3=OCH_3$
9: $R^1=H; R^2=NH_2; R^3=OCH_3$

Figure 2. 1-Aryl-*tetra*-hydro-β-carboline derivatives.

TABLE. The TPII-mediated cleavage of DNA induced by β-Carbolines **2**

Compound	DNA Cleavage Activity*	Intercalation
Azatoxin **1**	+ + + +	-
Etoposide (VP-16)	+ + + +	(+)
mAMSA	+ + + +	+ + +
3	+ +	-
4	+ + (+)	+
5	+ +	(+)
6	Inactive[12]	-
7	Inactive	-
8	(+)	-
9	(+)	-

*Relative DNA cleavage activity was determined. Each + indicates a 2- to 4-fold increase in activity as determined by densitometric analysis of cleavage assay gels. Each (+) indicates a 1- to 2-fold increase in activity.

The effect of the increased conformational mobility of the β-carboline framework compounds as compared to the rigid cyclic carbamate (azatoxin) and lactone systems (etoposide), is pronounced. The level of activity of compounds **8** and **9** and the potency of **5** compared to its azatoxin homolog demonstrates that these "flexible" β-carbolines are able to accommodate a more varied pendant ring substitution pattern relative to the etoposide[14] and azatoxin series[7]. Furthermore, activity is restored to the methylenedioxyphenyl nucleus [e.g. etoposide (+ + + +) vs. **3** (+ +) vs. *aza*-etoposide (inactive)[13]], thus indicating that loss of activity upon changing the

lactone to the cyclic carbamate is not electronic *or* stereoelectronic in nature. Finally, compounds **4** and **5** have the ability to intercalate as assessed by a topoisomerase I unwinding assay[15]; a result not seen with the more conformationally restrained compound azatoxin **1** (Table).

The variation in DNA cleavage patterns is also a reflection of the conformational mobility of these compounds (Figure 3). The β-carboline **4**, in addition to stimulating TPII-mediated DNA cleavage at unique sites, induces cleavage at sites similar to azatoxin, etoposide, and, to a lesser extent, mAMSA. In comparison, **5** only shows DNA cleavage at sites similar to mAMSA derivatives and **3**, **6**, and **8** show an independent and distinct pattern (data not shown). These results suggest that these conformationally mobile compounds associate with DNA inducing deformations recognized by the enzyme that are either unique (e.g.**3**, **4**, **8**, and **9**) or similar to those induced by known TPII-active agents (e.g. **4** and **5**). The nature of this deformation is profoundly influenced by the nature of the 1-aryl substituent and the type of functionality found on the pendant ring.

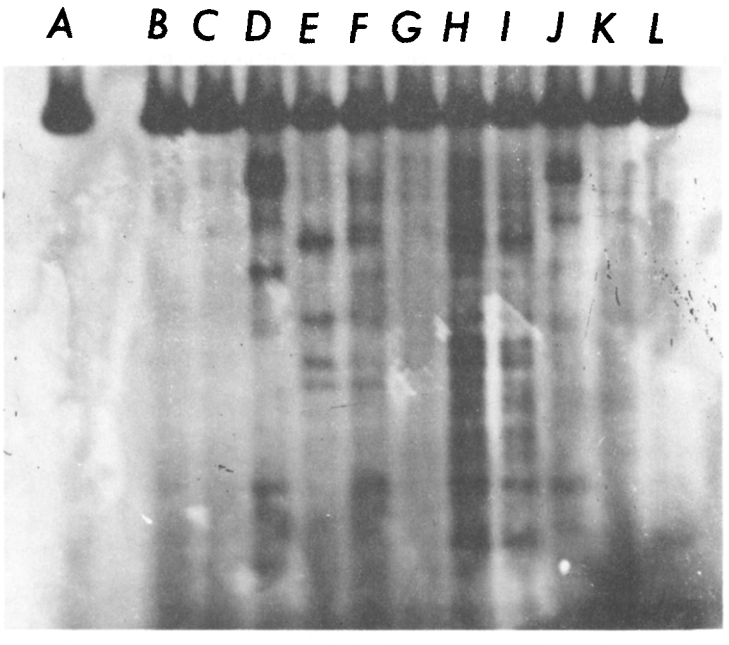

Figure 3. DNA double strand breaks induced by β-carboline derivative **6**in the presence of purified TPII fromhuman placenta. ^{32}P-End labeled pUC 18 plasmid DNA was incubated with drug and enzyme for 30 min. at 37° C. Reactions were terminated the addition of SDS, EDTA, and proteinase K (t0 final concentrations of 1%, 20 mM, and 80 μg/mL, respectively). DNA fragments were separated on a 1.4% agarose gel. *Lane A*, DNA; *Lane B*, DNA & TPII; *Lane C*, 1.0% DMSO; *Lane D*, 25μM mAMSA; *Lane E*, 100 μM etoposide; *Lane F*, 100 μM **1**; *Lane G*, 10 μM **4**; *Lane H*, 50 μM **4**; *Lane I*, 100 μM **4**; *Lane J*, 25 μM **5**; *Lane K*, 100 μM **5**; *Lane L*, 250 μM **5**.

Our model for the pharmacophore of TPII-active agents would hypothesize that the pendant group of the *tetra*hydro-β-carboline system resides in the DNA minor groove and that the pendant aryl 4'-substituent might serve as a hydrogen bond donor. This hypothesis requires that the dihedral angle between the aryl moiety and the pendant group (C4a-C9a-C1-C1') be able to closely approximate the dihedral angle observed in the rigid epipodophyllotoxin (-108°)[16] Molecular modelling calculations of **4** suggest that the C4a-C9a-C1-C1' dihedral angle would be -136° for a pseudoaxial pendant group and -108° for a pseudo-equatorial pendant group.[17] Interestingly, the pseudoaxial conformer is calculated to be the most stable by ≈7 Kcal/mol[18] and the energy for distortion of the minimum energy dihedral angle (-136°) to ≈-110° is small (< ≈2.5 kcal/mol) for **4**; allowing these compounds to access the dihedral angle necessary for activity in the epipodophyllotoxin nucleus with only a small expense in energy.

Furthermore, the mAMSA-like TPII-mediated DNA cleavage pattern of compounds **4** and **5** suggests that their pendant rings overlap with the anilino ring of mAMSA, which we propose to reside in the variable substituent domain of our pharmacophore model. This again places the pendant ring in the minor groove, but above the planar aromatic domain. The previously described conformational mobility of these compounds is sufficient to allow the pendant ring to mimic the C13-C14-N15-C16 dihedral angle (24°) of the anilino ring above the planar aromatic region mAMSA[19]. This dual mode of activity seems to be a result of the 1-indoyl-β-carbolines having a pseudo-C2-axis of symmetry that allows DNA association with the pendant ring either above or below the aromatic domain; thus effectively mimicking the active conformations of mAMSA, etoposide,and/or azatoxin. In contrast, the more structurally rigid compounds each have a single distinct mode of DNA association that leads to effective cleavable complex formation.

In conclusion, the 1-aryl tetrahydro-β-carboline skeleton represents a minimal prototype for the design of TPII inhibitors based on the podophyllotoxin nucleus. Further structural modifications on this nucleus, including addition of functionality in the variable substituent region, may result in antineoplastic agents of superior activity.

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